Organoid media formulation #6

The following components are required for media preparation:

Item	Vendor	Catalog #	Size	Website
Advanced DMEM:F12	Thermo Fisher	12634028	500 mL	thermofisher.com
HEPES	Thermo Fisher	15630080	100 mL	thermofisher.com
B-27 Supplement	Thermo Fisher	17504-044	10 mL	thermofisher.com
L-Glutamine	ATCC	30-2214™	100 mL	atcc.org
Dimethyl sulfoxide (DMSO)	ATCC	4-X™	25 mL	atcc.org
EGF	Bio-techne	236-EG	200 µg	bio-techne.com
Gastrin	Bio-techne	3006	1 mg	bio-techne.com
FGF-10	Bio-techne	345-FG	2x 25 µg	bio-techne.com
Nicotinamide	LKT Labs	N3310	50 g	lktlabs.com
N-acetyl cysteine	LKT Labs	A0918	10 g	lktlabs.com
TGF-beta 1	Bio-techne	240-B-010	10 µg	bio-techne.com
HA-R-Spondin1-Fc 293T (RSPO1) Conditioned Media	For each 500 mL of complete organoid media, 50 mL of RSPO1 conditioned media is required. Refer to vendors instructions to prepare conditioned medium from Trevigen Cultrex® HA-R-Spondin1-Fc 293T Cells (Trevigen Cat # 3710-001-01). The protocol for cell culture and conditioned medium generation is available at: https://trevigen.com/docs/protocol/protocol_3710-001-01.pdf			
CRL-2647 L Wnt-3A Conditioned Media	For each 500 mL of complete organoid media, 250 mL of WNT3A conditioned media is required. Refer to the product sheet for instructions to prepare conditioned medium from ATCC CRL-2647 L Wnt-3A cells. The protocol for cell culture and conditioned medium generation is available at: https://www.atcc.org/~/ps/CRL-2647.ashx			

Refer to the manufacturer of individual components for important safety and handling considerations.

Media preparation procedure

1. Thaw B-27 and L-Glutamine on ice or in a refrigerator at 2-8°C.
   Aliquot into working volumes and freeze. Do not re-freeze/thaw multiple times.

2. Briefly centrifuge the vials containing the EGF, Gastrin and FGF-10 (2 vials) to ensure the material is at the bottom of the vial.

3. Aseptically reconstitute the following components: EGF, FGF-10, and Gastrin according to the manufacturer's instructions in the recommended buffer listed in the table below. We recommend incubating in buffer for 15 minutes at room temperature.

Item	Size	Buffer	Volume of Buffer	Final Concentration
EGF	200 µg	Advanced DMEM:F12	2.0 mL	100 µg/mL
Gastrin	1 mg	Advanced DMEM:F12	4.7 mL	100 µM
FGF-10 (2 vials)	2 x 25 µg	Advanced DMEM:F12	2x 0.25 mL	100 µg/mL
TGF-B1	10 µg	4 mM sterile filtered HCl	1.0 mL	10 µg/mL

4. Aseptically weigh and prepare working solutions of Nicotinamide and N-Acetyl Cysteine in the recommended buffer. If N-Acetyl Cysteine is difficult to dissolve, periodic vortexing and incubation in a 37.0°C water bath can help the material enter solution.

Item	Weight	Buffer	Volume of Buffer	Final Concentration
Nicotinamide	5 g	Advanced DMEM:F12	41.0 mL	1 M
N-Acetyl Cysteine	2.5 g	Advanced DMEM:F12	61.0 mL	250 mM

5. Prepare the complete growth medium formulation (makes 500 mL):

Item	Volume	Final Concentration
Advanced DMEM:F12	171.5 mL	N/A
HEPES	5.0 mL	10 mM
L-Glutamine	5.0 mL	2 nM
B-27	10.0 mL	1X
EGF	0.25 mL	50 ng/mL
FGF-10 (2 vials)	2 x 0.25 mL	100 ng/mL
Gastrin	50.0 µL	10 nM
Nicotinamide	5.0 mL	10 mM
N-Acetyl Cysteine	2.5 mL	1.25 mM
RSPO1 conditioned media	50 mL	10%
WNT3A conditioned media	250 mL	50%

6. For the first 2-3 weeks of culture only, supplement the complete media with the prepared TGF-B1 to a final concentration of 5 ng/mL by adding 1 µL per 2 mL complete growth medium. After 2-3 weeks TGF-B1 is no longer included in the complete growth medium.

7. Once prepared, store complete medium at 2-8°C in the dark. Do not freeze and avoid extended exposure to light. Label with date of preparation and discard after 30 days.

8. When using the medium during culture, only warm the volume required.

9. Refer to the manufacturer's documentation for appropriate storage conditions and stability of individual components once reconstituted.

Notes

- Purity and activity levels of the various components can change from lot-lot. Refer to the manufacturer's Certificates of Analysis to ensure equivalent quality when using a new lot of material.

- We do not recommend deviating from the formulation or substituting components from different vendors.

- We recommend that solutions are prepared on the same day they are used. If the solutions must be stored, aliquot and freeze at -80°C or below and use within 30 days. Once reconstituted the components will lose activity over time and this can negatively affect performance of the medium.

Warranty and Disclaimer

ATCC® products are warranted for 30 days from the date of shipment, and is valid only if the product is stored and used according to the information included on this product information sheet. This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. ATCC does not warrant that such information has been confirmed to be accurate. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product.